

What is claimed is:

- 1/ A method for the expression of high yields of active protein-Ig fusions comprising culturing a host transformed with DNA encoding a desired protein-Ig fusion in a culture system having a low temperature of about 27° C to about 35° C.
- 5 2. The method of claim 2 wherein the temperature is about 27°C to about 32° C.
3. The method of claim 3 wherein said transformed host is first cultured at a temperature above about 33° C for a period of time sufficient to allow growth of said host.
4. The method of claim 1 wherein said protein-Ig fusion comprises a member of the TNF 10 receptor family.
5. The method of claim 3 wherein said TNF receptor family member is a lymphotoxin- $\beta$  receptor, TNFR-55, HVEM or a fragment thereof.
6. The method of claim 1 further comprising the step of recovering active protein-Ig fusions from said culture system by hydrophobic interaction chromatography.
- 15 7. The method of claim 1 wherein said culture system comprises insect or bacterial cells.
8. An active protein-Ig fusion obtained by culturing a host transformed with DNA encoding the fusion in a culture system having a low temperature of about 27° C to about 35 ° C.
9. The fusion of claim 8 comprising a member of the TNF family.
- 20 10. The fusion of claim 9 comprising LT- $\beta$  receptor, or a fragment thereof.
11. The fusion of claim 9 comprising HVEM, or a fragment thereof.
12. A method of making a pharmaceutical preparation comprising an active protein-Ig fusion said method comprising:
- (a) culturing a host transformed with DNA encoding the protein-Ig fusion in a culture 25 system having a low temperature of about 27° C to about 32 ° C, thereby expressing active protein-Ig fusions;
- (b) recovering active protein-Ig fusions from said culture system; and
- (c) combining the active protein-Ig fusions of step (b) with a pharmaceutically acceptable carrier.

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13. The method of claim 12 wherein the protein-Ig fusion comprises a member of the TNF family, or a fragment thereof.

14. The method of claim 13 wherein the protein-Ig fusion comprises lymphotoxin- $\beta$  receptor or a fragment thereof.

5 15. The method of claim 13 wherein the protein-Ig fusion comprises HVEM, or a fragment thereof.

16. A pharmaceutical preparation obtained by

(a) culturing a host transformed with DNA encoding the protein-Ig fusion in a culture system having a low temperature of about 27° C to about 32 ° C, thereby expressing active protein-Ig fusions;

(b) recovering active protein-Ig fusions from said culture system; and

(c) combining the active protein-Ig fusions of step (b) with a pharmaceutically acceptable carrier.

17. The pharmaceutical preparation of claim 16 wherein the protein-Ig fusion comprises a member of the TNF family.

18. The pharmaceutical preparation of claim 17 wherein the protein-Ig fusion comprises a lymphotoxin- $\beta$  receptor or a fragment thereof.

19. The pharmaceutical preparation of claim 17 wherein the protein-Ig fusion comprises HVEM, or a fragment thereof.

20 20. A method for the expression of high yields of active protein-Ig fusions comprising culturing yeast transformed with DNA encoding a desired protein-Ig fusion in a culture system having a low temperature of about 10° C to about 25° C.

21. The method of claim 20 wherein the temperature is about 15°C to about 20° C.

22. The method of claim 20 wherein said transformed host is first cultured at a temperature above about 30° C for a period of time sufficient to allow growth of said host.

23. The method of claim 20 wherein said protein-Ig fusion comprises a member of the TNF receptor family.

24. The method of claim 23 wherein said TNF receptor family member is a lymphotoxin- $\beta$  receptor or a fragment thereof.

30 25. The method of claim 20 further comprising the step of recovering active protein-Ig fusions from said culture system by hydrophobic interaction chromatography.

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26. An active protein-Ig fusion obtained by culturing yeast transformed with DNA encoding the fusion in a culture system having a low temperature of about 10° C to about 25 ° C.
27. The fusion of claim 26 comprising a member of the TNF family.
- 5 28. The fusion of claim 27 comprising LT<sub>B</sub> receptor, or a fragment thereof.
29. The fusion of claim 26 comprising HVEM, or a fragment thereof.
30. A pharmaceutical preparation comprising an active protein-Ig fusion having an Ig Fc domain and peptide chains, wherein the Ig Fc domain is altered thereby altering the rate of disulfide formation in the hinge region of said protein-Ig fusion.
- 10 31. The preparation of claim 30, wherein said Ig Fc domain is altered by replacing at least one cysteine residue with alanine.
32. A protein-Ig fusion comprising an Ig-Fc domain crosslinked to a peptide derived from the TNF family wherein at least one cysteine residue on the Ig-Fc domain is replaced with alanine.
- 15 33. The protein-Ig fusion of claim 32 wherein said peptide is derived from a lymphotoxin-β receptor.
34. A method of making a protein-Ig fusion comprising an Ig Fc domain crosslinked to a peptide derived from the TNF receptor family by mutagenizing at least one cysteine residue to an alanine, thereby increasing the yield of active forms of fusion expressed.
- 20 35. The method of claim 34 wherein said peptide is LTBR, and the cysteines at positions 101 and 108 are mutagenized to alanines.
36. An LTBR-Ig fusion protein comprising alanine at positions 101 and 108 of the LTBR peptide.